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ULTRASTRUCTURAL OBSERVATIONS ON THE PHAGOCYTIC UPTAKE OF FOOD MATERIALS BY THE CILIATED CELLS OF THE RHYNCHOCOELAN INTESTINE

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Previous observations at the light microscope level have shown that throughout the phylum Rhynchocoela digestion occurs by a combination of extra- and intracellular processes, with semi-digested food being taken up from the intestinal lumen for subsequent completion of digestion within intracellular vacuoles (Reisinger, 1926; Jennings, 1960, 1962; Gibson and Jennings, 1969; Jennings and Gibson, 1969). Only two cell types are present in the gastrodermis, namely pyriform gland cells whose secretions are responsible for the extracellular phase of digestion, and ciliated columnar cells in which the intracellular phase occurs. The intracellular vacuoles are relatively large (1–7 μ in diameter) and their occurrence within cells whose free distal border bears apparently normal and functional cilia poses the problem of the precise means of their formation and the method whereby materials destined for intracellular digestion enter the cells. Since the extracellular digestive phase generally reduces the food to a homogeneous semi-fluid consistency, the food vacuoles could conceivably develop from fusion of much smaller pinocytotic vesicles, which themselves formed between the bases of the cilia. That this is not the case, however, was shown by experiments in which starch grains ranging from 1–5 μ in diameter, and administered as components of test meals, appeared in the vacuoles quite unchanged and retaining their characteristic appearance when viewed by polarized light. Thus the ciliated cells can be regarded as truly phagocytic, using this term to denote intake of discrete masses large enough to be visible with the light microscope, and so far as is known this phenomenon is unique in the animal kingdom. Various other invertebrate groups possess intracellular digestion and often, as in numerous gastropod and lamellibranch molluscs, the digestive tissue also contains ciliated or flagellated components (Owen, 1966). In all these instances, though, the indications are that there is considerable structural and functional diversity amongst the cell types present, and that the ciliated cells are concerned with transporting or mixing the gut contents whilst actual uptake of particulate matter is effected by unciliated components of the gastrodermis.

In the present study, therefore, the rhynchocoelan gastrodermis has been investigated at the ultrastructural level, both in the resting condition between meals and during uptake of material from the intestinal lumen, to determine the precise means whereby particulate matter enters the columnar cells and to detect any peculiarities of the ciliated surface. Towards this last objective, comparative studies have also been made on the ciliated surfaces of the foregut, which are not concerned with digestion, and of the epidermis.

Materials and Methods

The rhynchocoelan selected for study was the heteronemertean *Lineus ruber* (O. F. Müller), a species whose digestive physiology has been studied previously by histological and histochemical methods (Jennings, 1960, 1962; Jennings and Gibson, 1969). Specimens starved for seven days were used for studies on the resting gastrodermis; others, starved for the same length of time, were fed on clotted avian blood and fixed at intervals of 15, 30 and 60 minutes after feeding to study the entry of materials into the gastrodermal cells. Blood (from the Japanese Quail *Coturnix coturnix japonica*) was used as the test food since hemoglobin has a characteristic appearance with the electron microscope, even when partially digested, and that of an avian species was selected due to the small size of the erythrocytes which on occasion permits their phagocytosis intact.

To prevent ejection of gut contents during fixation the rhynchocoelans were first lightly narcotized n 0.36 *M* magnesium chloride. They were then transferred to fixative at 4° C and cut into pieces 1–1.5 mm in length. Fixation, dehydration and embedding in Shell Epikote resin (epon 812) were based on the procedures of Manton and Parke (1965) and Parke and Manton (1967). Initial fixation was for 2 hours in 4% glutaraldehyde in 0.1 *M* cacodylate buffer at pH 7.0 and containing 0.25 *M* sucrose, followed by three rinses, each of 20 minutes duration, in buffer containing progressively reduced amounts of sucrose (0.25 *M*, 0.125 *M* and sucrose-free, respectively) and postfixation for 1 hour in 2% osmium tetroxide in sucrose-free cacodylate. All these procedures and subsequent dehydration in graded ethanols were carried out at 4° C.

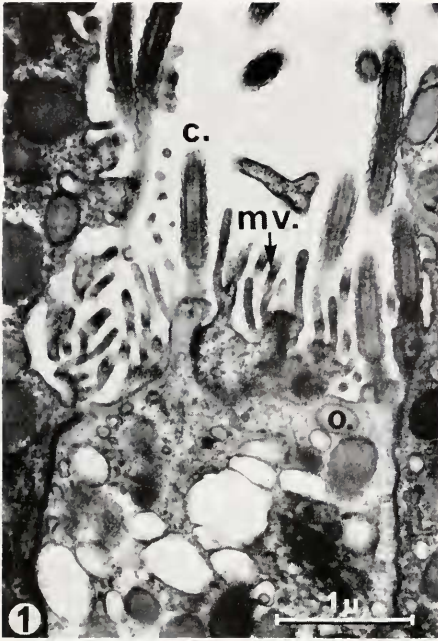
Silver to grey sections cut with glass knives on a Cambridge (Huxley) ultramicrotome and mounted on uncoated copper grids (200 mesh) were stained for 5 minutes in lead citrate (Reynolds, 1963) and examined in an AEI EM 6B electron microscope using a 50 μ or 25 μ objective aperture at 60 kv.

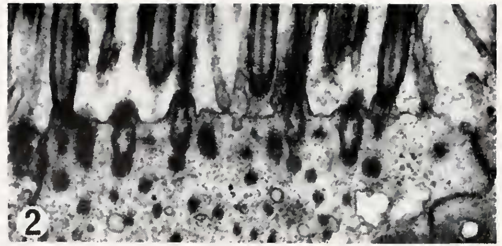
Other sections were cut at 0.5–0.1 μ, mounted on glass slides, stained with Azur II in borax (Jeon, 1965) and examined with the light microscope.

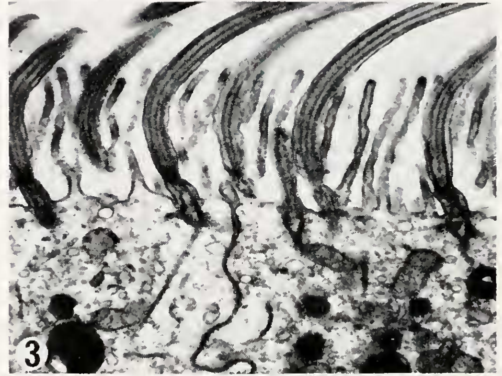
Attempts were made to visulaize phosphatase activity, which is a characteristic feature of the distal regions of cells concerned with food uptake in many animals, at the ultrastructural level in starved specimens by applying lead-salt replacement techniques of the type developed by Gomori (1939, 1952). For alkaline phosphatase Tranzer's (1965) method, as modified by Coleman, Evennett and Dodd (1967), was applied to glutaraldehyde fixed material which was then washed, postfixed in osmic acid, dehydrated and embedded in the standard manner. Small pieces of tissue were incubated for 1 hour at 20° C in a Gomori-type medium consisting of 0.1 ml sodium β-glycerophosphate, 6 ml 3% sodium barbitone, 1 ml 0.1 *M* lead nitrate and 1.1 ml trisodium citrate, with the final pH adjusted to 9.0 with barbiton. Inclusion of 0.1 ml 5% magnesium nitrate in the incubation medium, in pilot experiments, was found to enhance the reaction and this was eventually adopted as standard procedure.

Incubation was followed by washes in cacodylate buffer, 2% acetic acid and again in buffer, before osmication.

Acid phosphatase activity was demonstrated by a similar Gomori-type method, again based on a modification by Coleman, Evennett and Dodd (1967) of techniques described by Miller (1962) and Ericsson and Trump (1965). Incubation







FIGURES 1–4.

after glutaraldehyde fixation was for 1 hour at 20° C in a freshly filtered mixture consisting of 50 ml 3% sodium β-glycerophosphate and 500 ml of 0.05 M acetate buffer containing 0.6 g lead nitrate. Incubation was followed by washing in buffer and 2% acetic acid as before.

Controls for these methods consisted in each case of the incubation of comparable pieces of tissue in the standard medium devoid of the glycerophosphate substrate.

Observations

The distal region of the resting gastrodermis

In specimens of *Lineus ruber* starved for seven days the ciliated columnar cells of the gastrodermis are 80–100 μ tall and 6–8 μ wide. They are generally devoid of food vacuoles but possess distally a zone 10–15 μ in depth which is packed with oval bodies 0.4–1.0 μ long and 0.4–0.5 μ in diameter. These stain intensely with Azur II and at the ultrastructural level are seen to be bounded by a single membrane, often slightly crenellated, and to be moderately electron-dense after staining with lead citrate (Figs. 1, 4, and 5). A characteristic feature of the rhynchocoelan gastrodermis, as observed after application of histochemical methods, is the presence of a distal zone of alkaline phosphatase activity (Jennings, 1962; Jennings and Gibson, 1969) and in view of the similar distribution of these oval inclusions it was thought that this enzymic activity might reside in them. With the techniques employed here, however, the great majority of these bodies showed no reaction for either acid or alkaline phosphatases. Some 10–15% showed a slight reaction for alkaline phosphatase on their bounding membranes, but this did not approach the intense reaction obtained in the earlier light microscope histochemical studies in this region of the columnar cells.

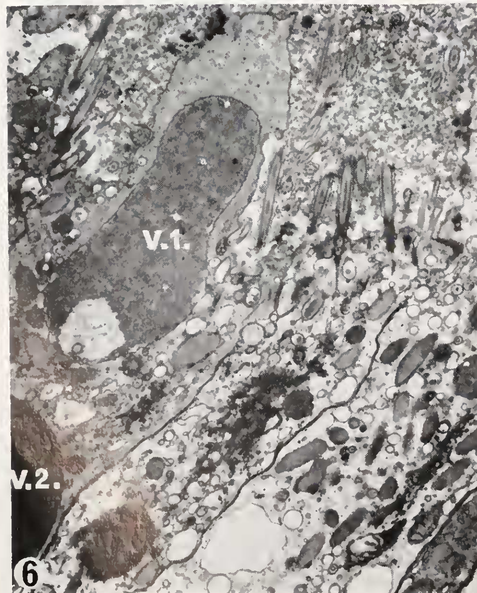
The free distal border of the cells bears flagelliform cilia, 15–18 μ long and 0.2–0.3 μ in diameter, which are distributed somewhat irregularly over the cell surface. The cilia are motile in life and ultrastructurally have the usual and characteristic internal arrangement of two central longitudinal fibers with nine other fibers arranged around them (Fig. 4). Apart from their greater length they are identical with the cilia of the foregut (Fig. 2) and epidermis (Fig. 3);

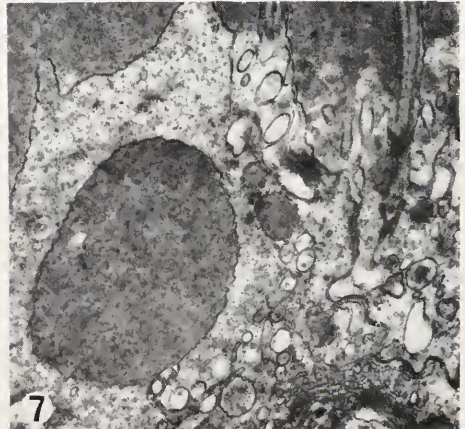
FIGURE 1. *Lineus ruber;* distal region of a ciliated columnar cell in the gastrodermis of a specimen starved for seven days before fixation; c., cilium; m.v., microvilli; o., transverse section through an oval body.

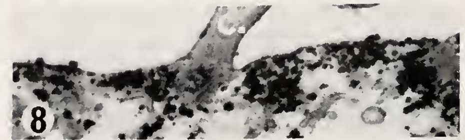
FIGURE 2. *L. ruber;* distal region of a ciliated cell in the foregut, showing cilia, basal bodies and microvilli; magnification as in Figure 1.

FIGURE 3. *L. ruber;* distal regions of two epidermal cells, showing cilia, a ciliary rootlet, microvilli and mitochondria; magnification as in Figure 1.

FIGURE 4. *L. ruber;* distal regions of three ciliated gastrodermal cells from a specimen fixed fifteen minutes after a blood meal. The intestinal lumen is filled with haemoglobin (h.) resulting from haemolysis of erythrocytes and one cell is putting out pseudopodia-like lamellae (lm.) to enclose a portion of the haemoglobin within a food vacuole. A cilium (c.) is visible adjacent to one of the lamellae and is clearly part of the same cell, whilst other cilia can be seen in transverse section amongst the gut contents. A completed vacuole (v.) with compacted and denser contents, is present deeper within the cell. The cells also show the oval bodies (o.), which are a characteristic feature of this region of the gastrodermis, and microvilli; magnification as in Figure 1.







FIGURES 5–8.

the basal bodies and cross-striated ciliary rootlets show no anomalous features, and the entire ciliary structure resembles that described in all other animal groups possessing ciliated cells.

Although no differences in structure could be found in the cilia of the gastrodermis, foregut and epidermis, there is a marked difference in their relative densities on the cell surfaces, the gastrodermal cilia being significantly fewer in number per cell as compared with the other two types and consequently much less closely packed. Thus gaps of 2–3 μ or more occur between the gastrodermal cilia throughout the length of the intestine, whereas in the foregut the cilia are extremely regular in their arrangement and occur at intervals of only 0.1–0.15 μ.

The cell surface between the gastrodermal cilia is produced into irregularly distributed microvilli, each 0.1 μ in diameter and 0.5–0.6 μ long (Fig. 1). The microvilli possess an external granular coating, or glycocalyx, but show no internal differentiation into a central core and an outer layer such as occurs in the gut microvilli of some other animal groups. They are uniform cylindrical structures and are usually orientated at right angles to the cell surface and parallel with the cilia, but sometimes they occur at varying angles and in a few instances are dichotomous, splitting into two equal sized structures 0.1 μ from the base. The microvilli are particularly noticeable in starved specimens, in which the intestinal lumen is empty.

Similar microvilli occur between the cilia of the foregut (Fig. 2) and epidermis (Fig. 3).

Uptake of food by the gastrodermis

The gastrodermal gland cells discharge their proteolytic secretions when a test meal of quail erythrocytes enters the intestine. Haemolysis, rupture of nuclear membranes and release of nuclear contents occur extremely rapidly and sections of *L. ruber* fixed fifteen minutes after feeding show the intestinal lumen to contain a granular, fairly homogeneous mass. The cilia and microvilli retain their identity and appear as in the resting gastrodermis, but the plasma membrane between these structures shows bud-like protuberances which rapidly extend outwards into the lumen contents and develop into pseudopodia-like lamellae (Figs. 4 and 5). These lamellae vary enormously in size and may eventually reach 6 μ in length. They generally arise at distances of 1–5 μ from each other and as

FIGURE 5. *L. ruber;* distal region of a ciliated gastrodermal cell thirty minutes after a blood meal, showing two lamellae which have almost completed formation of a food vacuole. The lamellae are each flanked by a single cilium (c). o., oval body seen in longitudinal section; magnification as in Figure 1.

FIGURE 6. *L. ruber;* distal portions of three ciliated gastrodermal cells, thirty minutes after feeding. The cell to the left shows a recently completed vacuole (v.1.), flanked by a cilium, and below this is an earlier vacuole (v.2.) with much denser contents. The other cells show cilia and microvilli; magnification one half of Figure 1.

FIGURE 7. *L. ruber.* Portion of the distal region of a ciliated gastrodermal cell, fifteen minutes after feeding, showing a recently completed vacuole. Magnification as in Figure 1.

FIGURE 8. *L. ruber.* Extreme distal region of a ciliated gastrodermal cell in a specimen starved for seven days. The black granules represent sites of alkaline phosphatase activity. c., cilium. Tranzer's method, as modified by Coleman, Evennett and Dodd. Magnification as in Figure 1.

they extend outwards into the gut lumen tend to curve downwards somewhat, back towards the cell surface. Eventually the tips of two lamellae meet and fuse, trapping a mass of semidigested food between themselves and the cell surface (Figs. 6 and 7). In this way a vacuole up to 5 or 6 μ in diameter, containing semidigested food and bounded by a single membrane, is formed and this passes back into the cell for subsequent intracellular digestive processes. As the newly formed vacuole moves away from the cell surface more lamellae develop and the entire process is repeated several times. Thus within thirty minutes of feeding most of the ciliated gastrodermal cells show two or more vacuoles in their distal regions (Figs. 4 and 6). The contents of the vacuoles become compacted and denser in appearance as they pass deeper into the cells, presumably as water is absorbed and digestion begins.

Throughout this process the gastrodermal cilia remain distinct, and clearly are not concerned in any way with the uptake of materials from the lumen. In many instances the origin of a lamella lies extremely close to a cilium (Figs. 4 and 5), but it is always distinct from any part of the ciliary apparatus. The microvilli are often less distinct during the phagocytic process and were in fact absent from many fields examined, especially in sections of rhynchocoelans fixed thirty or sixty minutes after a meal. When clearly visible they showed no significant differences in structure or disposition as compared with their appearance in the resting gastrodermis.

No evidence was found to suggest that the food vacuoles ever form by invagination and vesiculation of the plasma membrane and it would appear, therefore, that the entry of visible food materials into the ciliated columnar cells is the result of a normal phagocytic process, with pseudopodia-like lamellae protruding from the cell surface between the cilia to engulf the food and enclose it within a typical food vacuole.

Phosphatases in the ciliated cells

Throughout the gastrodermis a positive reaction for alkaline phosphatase was obtained in the extreme distal region of the ciliated columnar cells, the reaction being concentrated in a narrow band 0.1–0.2 μ in depth immediately below the plasma membrane (Fig. 8). A much more scattered and diffuse reaction was obtained in the cells to a depth of 20–30 μ from the distal region. In neither case was the reaction associated with any organelle, apart from the slight reaction mentioned earlier on the limiting membrane of a small proportion of the oval bodies found in this region of the cell. Only starved *L. ruber* were used in this part of the study, and no observations were made on the occurrence or distribution of alkaline phosphatase during phagocytosis.

The only reaction obtained for acid phosphatase was in lysosomes occuring in the basal portions of the ciliated cells in the region of the nuclei.

Discussion

The evidence presented here shows that the food vacuoles of the rhynchocoelan gastrodermis result from a truly phagocytic process, in which temporary outgrowths of the cell surface meet to enclose material lying in the gut lumen. The

vacuole formed in this way then passes back into the gastrodermal cell for subsequent intracellular digestion of its contents. This phagocytic process occurs between the cilia of the gastrodermal cells and is quite independent of them. The only modification apparent in the gastrodermal cilia which can be construed as related to phagocytosis is their relatively sparse distribution over the cell surface, when compared with the more densely packed cilia of the foregut and epidermis. This, presumably, is to leave adequate surface area available for development of the phagocytic outgrowths at the appropriate stage of the digestive process. The greater length of the gastrodermal cilia, relative to those of either the foregut or epidermis, may well in turn be an adaptive feature which compensates for this reduction in the number of cilia present per unit area of cell surface.

An earlier interpretation of this phenomenon of phagocytosis by ciliated cells, based entirely on observations with the light microscope, was that the cilia themselves somehow coalesced into pseudopodia-like structures which engulfed material from the gut lumen (Jennings, 1960). A re-examination of the material used in the earlier study, in the light of the present work, shows that the apparent coalescence of the cilia at the time when food vacuoles are appearing in the gastrodermis is a fixation artefact, adjacent cilia and cell outgrowths having clumped together into structures whose precise nature cannot be resolved by the light microscope.

The microvilli which occur between the cilia of the gastrodermis, foregut and epidermis are a fairly common feature of ciliated epithelia and have been described in a variety of tissues from both invertebrate and chordate groups (Fawcett and Porter, 1954; Porter and Bonneville, 1964; Iwai, 1967a, 1967b). The gastrodermal microvilli in the rhynchocoelan are probably concerned with absorption of simple soluble materials which are either present initially in the food or produced during the first, extracellular, phase of digestion. Ugolev (1960, 1965) has presented sound evidence for the participation of the microvilli of the vertebrate intestine in the actual digestive process, as contact catalysts ensuring intimate mixing of enzymes and substrates, and there are indications that they play the same role in some invertebrates such as digenetic trematodes (Halton, 1966) and nematodes (Colam, 1969). It seems unlikely, however, that the microvilli of the rhynchocoelan gastrodermis can be at all effective in this way, since, like the gastrodermal cilia, they are sparsely distributed over the cell surface and do not occur as the tightly packed and regularly arranged outgrowths seen in other animal groups.

The role of the foregut microvilli remains obscure. There is no digestion in this part of the gut in any rhynchocoelan so far studied and food does not remain in it for more than a few seconds (Jennings and Gibson, 1966). It is difficult to see, therefore, any possible role for these microvilli unless there is absorption here of excess water taken in through the mouth with the food.

The microvilli of the rhynchoelan epidermis were first described by Fisher and Cramer (1967) in *Lineus ruber*. These authors also demonstrated absorption of glucose and amino acids across the epidermis and concluded that the microvilli are involved in this process. Jennings and Gibson (1969) reported the presence of non-specific esterase activity in the epidermis of *L. ruber* and suggested

that the enzyme responsible may be concerned with extra-corporeal digestion of simple proteins or polypeptides, the products then being absorbed by the mechanism described by Fisher and Cramer. In this connection it is interesting to note that Jennings and Gibson also found non-specific esterase activity in the foregut of *L. ruber,* at sites and in quantities comparable to those in the epidermis. Thus the same sort of link may exist in the foregut, between microvilli, non-specific esterase activity and absorption as is suggested for the epidermis.

The alkaline phosphatase activity found in the distal regions of the columnar cells and the acid phosphatase localized in lysosomes presumably correspond to the enzymic activities visualized in the cells in earlier, histochemical, studies (Jennings, 1962; Jennings and Gibson, 1969). Their precise roles in the intracellular digestive processes are at present under investigation at the ultrastructural level and will be reported in a later account.

Summary

1. Ultrastructural observations on the rhynchocoelan *Lineus ruber* confirm that the ciliated columnar cells of the gastrodermis are truly phagocytic, in that they engulf visible particulate material from the intestinal lumen, enclose it in food vacuoles and pass these back into the cell for subsequent intracellular digestion.

2. The cilia have the form and structure that is ubiquitous for these organelles throughout the animal kingdom and they are not concerned in any way with the phagocytic uptake of material from the gut lumen.

3. After the initial extracellular digestion, which renders the food semi-fluid and relatively homogeneous, the cell surfaces between the cilia develop pseudopodia-like outgrowths or lamellae which extend out into the gut lumen. The tips of two adjacent lamellae fuse to form a vacuole enclosing semi-digested food and the vacuole then moves back into the cell for completion of digestion.

4. Lamellae are only present when semi-digested food is present in the intestine and they are absent from the resting gastrodermis between meals.

5. Microvilli occur between the gastrodermal cilia and are believed to be concerned with absorption of simple soluble substances from the gut lumen. They also occur between the cilia of the epidermis and of the foregut.

6. A zone of alkaline phosphatase activity, not associated with any particular organelle, occurs in the extreme distal region of the columnar cells. Acid phosphatase activity can be demonstrated in lysosomes occurring in the basal regions of the cells.

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